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Comparative Transcriptomics of Early Meiosis in *Arabidopsis* and Maize☆

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ABSTRACT

Though sexually reproductive plants share the same principle and most processes in meiosis, there are distinct features detectable. To address the similarities and differences of early meiosis transcriptomes from the dicot model system *Arabidopsis* and monocot model system maize, we performed comparative analyses of RNA-seq data of isolated meiocytes, anthers and seedlings from both species separately and *via* orthologous genes. Overall gene expression showed similarities, such as an increased number of reads mapping to unannotated features, and differences, such as the amount of differentially expressed genes. We detected major similarities and differences in functional annotations of genes up-regulated in meiocytes, which point to conserved features as well as unique features. Transcriptional regulation seems to be quite similar in *Arabidopsis* and maize, and we could reveal known and novel transcription factors and *cis*-regulatory elements acting in early meiosis. Taken together, meiosis between *Arabidopsis* and maize is conserved in many ways, but displays key distinctions that lie in the patterns of gene expression.

KEYWORDS: Meiosis; Transcriptome; RNA-seq; Meiocytes; *Arabidopsis*; Maize

INTRODUCTION

Meiosis is a specialized cell division, and one of its most important characteristic is homologous recombination. Meiotic recombination leads to unique redistribution of the parental genomes and creates novel alleles and allele combinations, making it one of the underlying principles for developing new varieties in breeding. Initiation of meiotic recombination occurs during early meiosis, namely leptotene, the first stage of prophase I, in which programmed double-strand breaks are formed and homologous chromosomes start to pair and synapse (Padmore et al., 1991; Armstrong and Jones, 2003; Sheehan and Pawlowski, 2012). Meiotic processes in a wide range of organisms show similarities and differences. Within the scope of this paper, we focus on *Arabidopsis* (*Arabidopsis thaliana*) and maize (*Zea mays*) − two species that share key processes in meiosis, but possess distinct features.

The most obvious differences between *Arabidopsis* and maize are their size (both in phenotype and genome), their usefulness to humankind (weed *vs*. staple crop), and, connected with that, their distinct evolutionary trajectory until

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they reached their present form. The dicot plant *Arabidopsis* is a weed and underwent natural selection and speciation, resulting in many ecotypes adapted to certain areas and climates (Gaut, 2012). Maize, on the other hand, is a monocot plant whose wild ancestor, teosinte, was developed into the current inbred and hybrid lines by intensive breeding (Yamasaki et al., 2007). The most striking differences in the genomes between *Arabidopsis* and maize are their size (~125 Mb in *Arabidopsis vs.* ~2300 Mb in maize) (Arabidopsis Genome Initiative, 2000; Schnable et al., 2009), gene density (4 genes/100 kb in *Arabidopsis vs.* 2.3 genes/100 kb in maize) (Arabidopsis Genome Initiative, 2000; Haberer et al., 2005), and proportion of transposable elements (TEs; ~14% of *Arabidopsis* genome *vs.* ~85% of maize genome) (Okamoto and Hirochika, 2001; Schnable et al., 2009).

A further difference between *Arabidopsis* and maize with impact on this study pertains to the available published and genetic resources: A PubMed search for scientific literature resulted in 42,262 hits for the keyword "*Arabidopsis*" (or "*Arabidopsis thaliana*"), and 29,367 for "maize" (or 22,618 for "*Zea mays*") at the beginning of June 2013. *A. thaliana* is used as a model organism to decipher genes and pathways, and many resources and methods (like T-DNA mutant libraries and transformation) are firmly established. Maize, on the other hand, has had its research focused on breeding and cytogenetics for a long time, and this is reflected in the quality and quantity of gene annotations. The KEGG (Kyoto Encyclopedia of Genes and Genomes), for example, has 27,464 entries for *Arabidopsis*, *vs.* 22,420 for maize, which has more genes than *Arabidopsis* − the latest genome releases contain ~27,000 protein-coding genes for *Arabidopsis* (TAIR10) and ~39,000 protein-coding genes for maize (Version 5).

General differences in meiosis are the duration (~1 day in *Arabidopsis vs.* ~6 days in maize) (Hsu et al., 1988; Armstrong et al., 2003) and the kind of cytokinesis (simultaneous, meaning only once after meiosis II in *Arabidopsis vs.* successive, meaning twice, after meiosis I and II in maize) (Staiger and Cande, 1991; Peirson et al., 1997). In addition, the type of inflorescence is distinct between *Arabidopsis* and maize (bisexual *vs.* unisexual, respectively).

However, since the structure of the anthers and the processes during early male meiosis are very similar between *Arabidopsis* and maize, we decided to examine gene expression similarities and differences between them. Previously, we reported the transcriptome profile of *Arabidopsis* male meiocytes mixed from all meiosis stages (Chen et al., 2010) and a detailed analysis of only early male meiocytes from maize (Dukowic-Schulze et al., submitted). In this study, we compare the data from maize with new data from *Arabidopsis* which were generated with only early prophase stages. Since both datasets have been created in our labs, using the CCM method (capillary collection of meiocytes) (Chen and Retzel, 2013) for the isolation of meiocytes, the absence of differences in experimental procedures facilitates a direct comparison. In this study, we focused on the genes up-regulated in male meiocytes without paying attention to down-regulated genes.

RESULTS

Global comparison of transcriptomes between *Arabidopsis* and maize

To get a general overview of RNA-seq data from *Arabidopsis* and maize, we examined the RNA-seq datasets to determine which genome features the sequence-reads generally mapped to (Table 1). This comparison demonstrates that *Arabidopsis* and maize gene expression patterns have the same tendencies (Fig. 1A): The biggest proportion of reads maps to genes, and in relation, the number of reads mapping to annotated genes is the smallest in meiocytes, higher in anthers, and the highest in seedlings (90.9%, 95.2%, 97.5% in *Arabidopsis*, 80.0%, 85.8%, 87.3% in maize). Reads mapping to unannotated features and to mitochondrial sequences show an opposite trend: most of them are in meiocytes, fewer in anthers and the least in seedlings (5.2%, 3.8%, 1.9% in *Arabidopsis*, 17.0%, 11.7%, 10.9% in maize for unannotated features; 2.979%, 0.052%, 0.011% in *Arabidopsis* 0.180%, 0.006%, 0.002% in maize for mitochondria). Looking at the proportions of mapped reads from our previous *Arabidopsis* RNA-seq datasets shows far more reads mapping to unannotated features (Fig. 1A), attributable to improvements in annotation (TAIR9 *vs.* TAIR10) and data alignment pipelines.

Since most reads aligned to genes, we analyzed the gene expression patterns in meiocytes, anthers and seedlings in detail in *Arabidopsis* and maize. To get an overview of the number of genes expressed above certain thresholds, we examined the mean gene expression levels in all three samples in reads per million (RPM). The expression levels of interest were ≥2, 5, and 10 RPMs in both species. To make the results more comparable between *Arabidopsis* and maize, they were converted to a ratio based on the total numbers of genes queried for in *Arabidopsis* or maize (33,602 and 39,656, respectively, predicted genes from current genome releases). Applying this criterion, *Arabidopsis* had a larger fraction of highly expressed genes than maize in each sample, i.e. ~8.1%, 4.4%, and 2.9% (≥2 RPM), or ~14.3%, 16.8%, 3.7% (≥10 RPM) in meiocytes, anthers and seedlings respectively (Fig. 1B). Most genes expressed were present in seedlings for both *Arabidopsis* and maize, followed by anthers, and finally meiocytes. Many expressed genes were common to meiocytes and anthers, which is expected since anthers contain meiocytes within them. However, maize showed more expressed genes in common between meiocytes and anthers if the criteria ≥10 RPM was employed than in the case of ≥2 RPM (Fig. 1C). This signifies that when only higher-expressed genes were taken into account, the conditions became more restrictive and conclusions more valid: Using ≥10 RPM as an expression threshold, *Arabidopsis* and maize show quite similar gene expression patterns.

Samples were further analyzed for significantly differentially expressed genes using the DEseq package for *R* Statistical Analysis (Anders and Huber, 2012). This enables detection and visualization of genes that are significantly expressed at different levels between samples. Looking at the

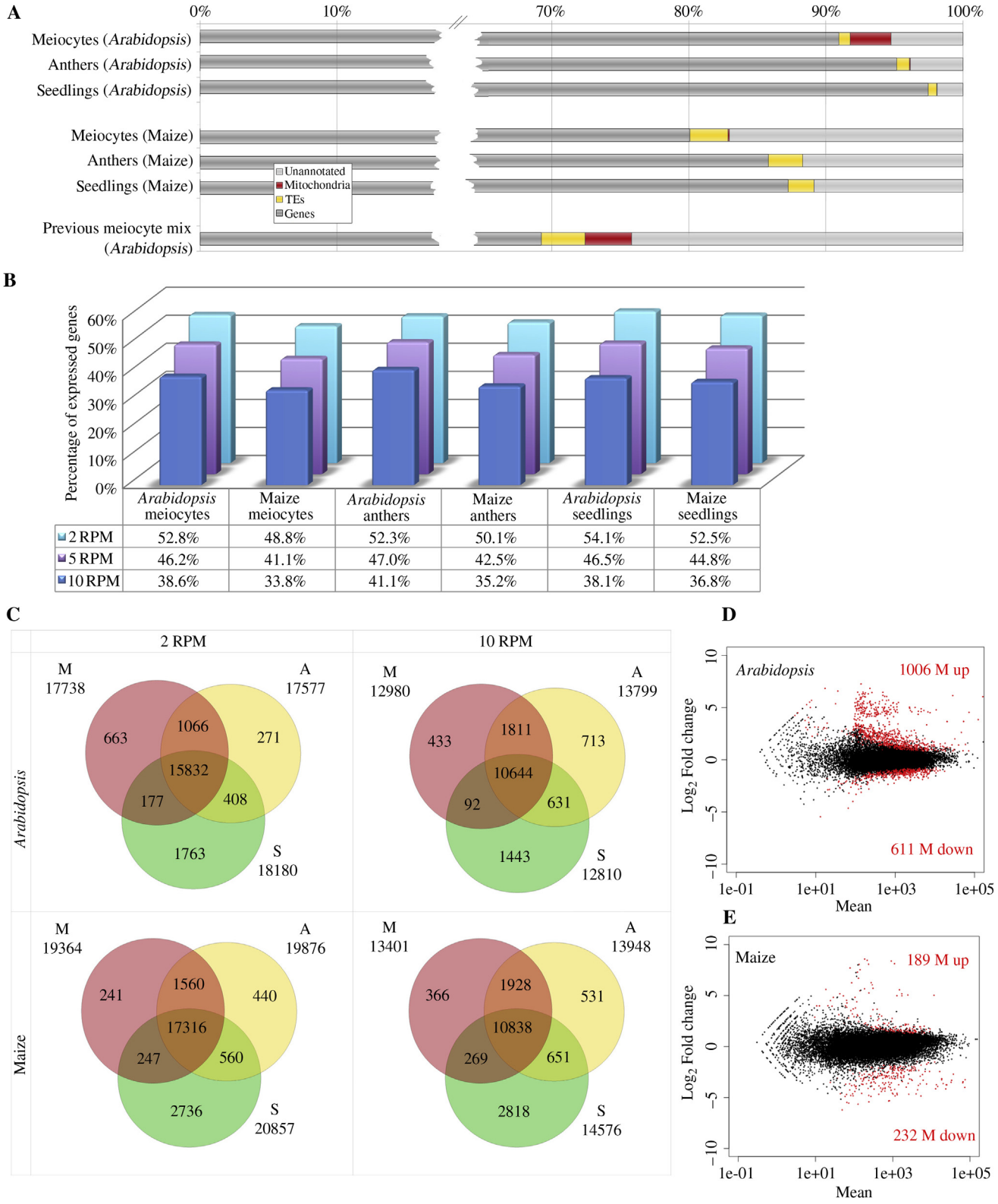

Fig. 1. Global transcriptome characteristics of *Arabidopsis* and maize meiocytes.

A: Proportion of reads mapping to nuclear genes, mitochondrial genes, TEs or unannotated regions. **B:** Proportion of expressed genes per sample relative to the number of total genes. **C:** Venn diagrams of expressed genes, showing separate and overlapping expression between samples. Meiocytes (M) in red, anthers (A) in yellow, seedlings (S) in green. **D** and **E:** Differentially expressed genes between meiocytes and anthers. Genes with significant differences in expression (adjusted *P*-value ≤ 0.05) in red, others in black. Numbers indicate the count of genes up- or down-regulated in meiocytes (M up, M down).

expression level of genes in meiocytes *vs*. anthers indicated far more differentially expressed genes in *Arabidopsis* than in maize (Fig. 1D and E). In addition, whereas *Arabidopsis* had more genes up-regulated in meiocytes, maize had more genes down-regulated in meiocytes (adjusted *P*-value $\leq$ 0.05, Fig. 1D and E).

Overall, the results of the analyses were dependent on the restrictions imposed on expression patterns. Whenever a threshold of only 2 RPM was applied, *Arabidopsis* and maize showed high numbers of expressed genes (Fig. 1C), and quite similar percentage of expressed genes (Fig. 1B). However imposing greater restrictions with a threshold of 10 RPM might be better suited to reveal differences (e.g., lower percentage of expressed genes in maize in meiocytes and anthers, but not in seedlings, Fig. 1B) and similarities (e.g., similar numbers of genes expressed in all three samples between *Arabidopsis* and maize, Fig. 1C).

Analysis with orthologous gene pairs

Expression profiles of *Arabidopsis* and maize show low correlation

To directly compare gene expression patterns in *Arabidopsis* and maize, we generated a list of pairs of orthologous genes (homologs between organisms). As previously seen in separate analyses, meiocyte and anther gene expression are closely related within species, showing high correlations (Fig. 2A) and similar expression profiles (Fig. 2B and C). However, there is a high level of divergence between organisms, especially apparent in clusters 4−10 in Fig. 2B. To facilitate detection of similarities between *Arabidopsis* and maize samples, we removed clusters 4−10 and repeated the analysis, which resulted in ten new clusters (Fig. 2C). Removing clusters did not produce a closer relation of all respective samples from both organisms, although the seedling columns were now moved closer to each other. Overall, gene expression patterns of putative orthologs seem to be different in most cases between *Arabidopsis* and maize.

Known meiotic genes are enriched in special clusters

Since studies on meiosis would benefit from good indicators for novel meiotic gene candidates, we examined if there are clusters in which known meiotic genes are concentrated. For this, we composed a list of known meiotic genes from *Arabidopsis*, most of them well-characterized, based on previous publications (Chen et al., 2010; Yang et al., 2011), and added their putative maize homologs. Out of 46 meiotic genes which are preferentially expressed in meiocytes, 5 were not included in our cluster data. Of the remaining 41 genes, 34 were contained in cluster 1 of Fig. 2B. They were more spread out in the clusters in Fig. 2C, but still enriched in clusters 1 (17 genes), 2 and 7 (8 genes each). A separate clustering using only the 46 meiotic genes clearly shows the up-regulated expression in meiocytes or anthers *vs*. seedlings in both *Arabidopsis* and maize (Fig. 2D). In this clustering of known meiotic genes, the seedling samples are more closely related to each other than to meiocytes and anthers from the same species, clearly demonstrating the similarity of meiotic gene expression between *Arabidopsis* and maize. The meiotic genes *MMD1*, *DMC1* and *SDS* are pointed out as examples (Fig. 2D).

Genes up-regulated in meiocytes show functional differences and similarities

To select orthologous gene pairs that are up-regulated in meiocytes *vs*. seedlings in both organisms, we used the DEseq package for *R* Statistical Analysis as described in Material and Methods. Similarities and differences were revealed when we performed functional annotation for gene ontology (GO) categories using AgriGO and Revigo (Du et al., 2010; Supek et al., 2011). Intriguingly, the scatterplots in Fig. 3A and B derived from orthologous genes, using either the *Arabidopsis* gene IDs (Fig. 3A) or the maize gene IDs (Fig. 3B), resulted in striking differences regarding biological processes: first of all, far more significantly enriched GO terms were found for *Arabidopsis* than for maize (194 *vs*. 51 with Revigo, 64 *vs*. 0 with AgriGO). In addition, terms for reproduction, such as meiosis and flower organ development, are abundant in the analysis *via Arabidopsis* gene IDs while they are absent in the analysis *via* maize gene IDs, which has heavier focus on metabolism. However, similarities are also present, especially with respect to transcriptional regulation.

Genes that were up-regulated in meiocytes *vs*. seedlings in only *Arabidopsis* or maize demonstrate further differences but also have some functional similarities (Fig. 3C and D). Significantly enriched processes in both species are organelle organization, protein import/targeting/localization to organelle, DNA repair and double-strand break repair. Processes exclusive to *Arabidopsis* meiocytes include silencing, DNA modification and packaging, and terms related to the cell cycle. Processes exclusive to maize meiocytes concentrate on metabolism and now also on glycosylation.

Overall, there appear to be more differences than similarities in the meiocyte transcriptomes between *Arabidopsis* and maize, even when using putative orthologous genes for analysis.

Transcription factors up-regulated in meiocytes

Because “regulation of transcription” was one of the few biological processes found up-regulated in meiocytes by analysis *via* both *Arabidopsis* gene IDs and maize gene IDs (Fig. 3B and C), we examined transcription factors in more detail. We selected all genes in the GO categories GO:0003700 (transcription factor activity) and GO:0030528 (transcription regulator activity). Although the analysis had been done with orthologous gene pairs, we did not obtain the same number of genes in these GO categories, due to annotation differences: The 17 transcription factors *via Arabidopsis* gene IDs and 12 transcription factors *via* maize ID are listed in Table 1. The transcription factor families were then assigned using annotations from the Plant Transcription Factor Database (PlnTFDB) (Riaño-Pachón et al., 2007). The transcription factor families with the most members up-regulated in meiocytes are MADS-box transcription factors, basic Helix-loop-Helix (bHLH) transcription factors, and

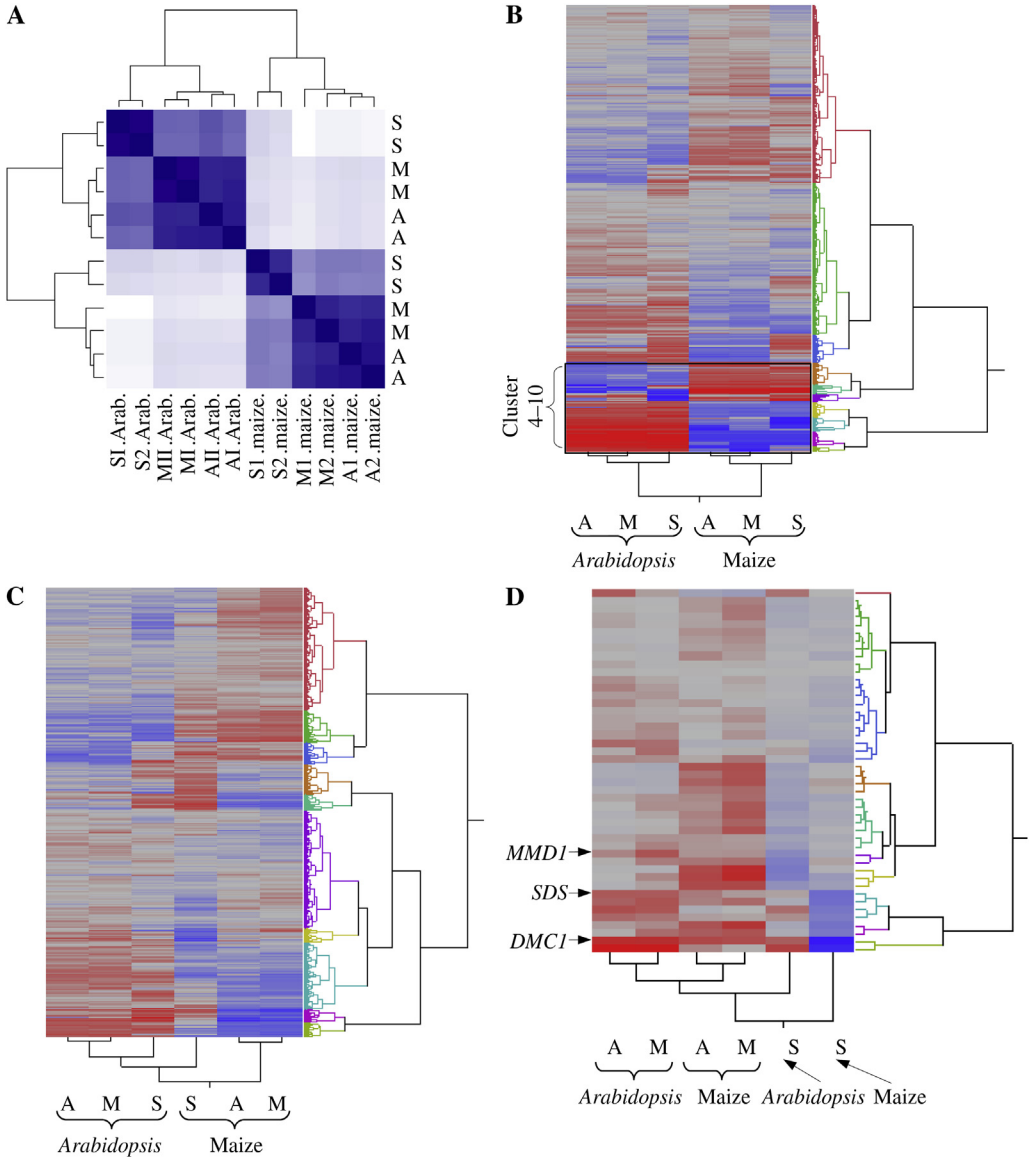

Fig. 2. Correlation and expression heatmaps of orthologous genes from *Arabidopsis* and maize.

A: Correlation dendrogram of all samples. Generated with *R* Statistical Analysis, using orthologous gene data. **B:** Expression heatmap of upper 70 percent quantile of orthologous genes. Samples related best within species. **C:** Expression heatmap of clusters 1−3 from panel **B**. Meiocytes (M) and anthers (A) within an organism are closest related, seedlings (S) show more similarity to each other than in panel **B**. **D:** Expression heatmap of meiocyte-preferential expressed meiotic genes. M, meiocytes; A, anthers; S, seedlings; red represents high expression, blue represents low expression. Values are in $\log_2$ scale of normalized reads. Data was clustered into 10 clusters each with the Ward method. Clusters in different colors, red = #1, green = #2, blue = #3, orange = #4, mint = #5, purple = #6, ocre = #7, turquoise = #8, pink = #9, yellow-brown = #10.

basic-leucine zipper (bZIP) transcription factors (Fig. 4A). Well-known examples for the MADS-box transcription factors included in our data are APETALA3, PISTILLATA, AGAMOUS and AGAMOUS-Like (AGL) MADS-box proteins (Bowman et al., 1991; Rounsley et al., 1995; Mizukami et al., 1996). Further prominent transcription factors found here are AMS (ABORTED MICROSPORES), involved in tapetal development, and CRC (CRABS CLAW), involved in carpel development (Bowman and Smyth, 1999; Sorensen et al., 2003). We performed a hierarchical clustering of transcription factors up-regulated in meiocytes for the annotated transcription factors and found opposite expression pattern in the putative *AGAMOUS* ortholog gene pair (Fig. 4B, black print). We then went back to the complete ortholog list, and found three more putative maize orthologs for AtAGAMOUS which show more conserved expression pattern (Fig. 4B, grey print).

There were many more transcription factors up-regulated only in *Arabidopsis* (291) or only in maize (57). For those annotated in the Plant TFDB, the family distribution shows similar amounts of MADS-box, bHLH, and bZIP transcription factors between *Arabidopsis* and maize (Fig. 4A). Additionally a pronounced peak for AP2-EREBP was found in both species which is a transcription factor unique to plants and typically involved in development (Riechmann and Meyerowitz, 1998).

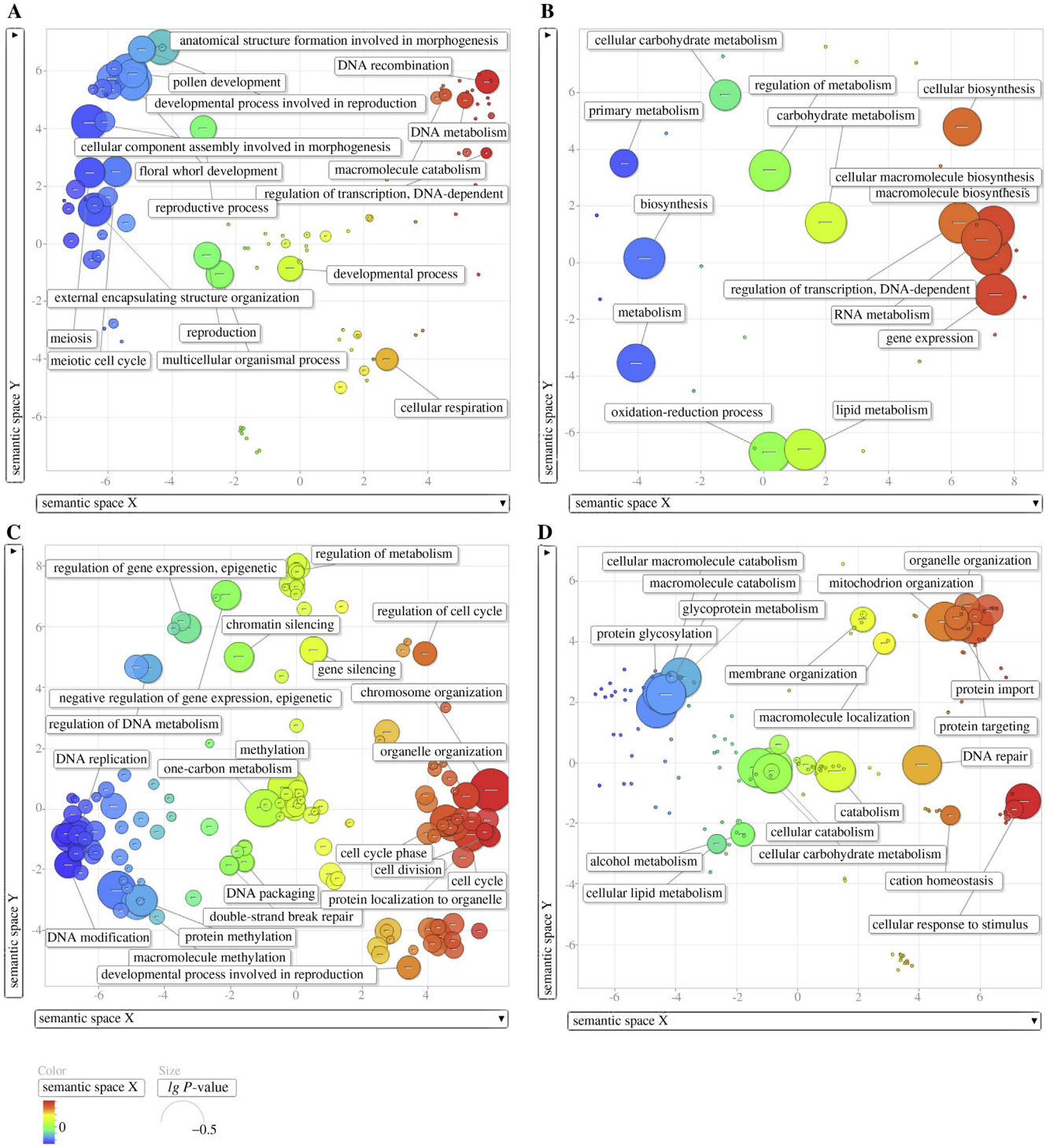

Fig. 3. Scatterplots of biological processes enriched in meiocytes *vs*. seedlings.
Analysis of orthologous genes up-regulated in meiocytes in both *Arabidopsis* and maize, *via Arabidopsis* gene IDs (**A**) and *via* the corresponding maize gene IDs (**B**). Analysis of genes significantly up-regulated in meiocytes in only *Arabidopsis* (**C**) or only in maize (**D**). The scatterplots were generated by Revigo, arranging biological process terms in semantic space. Colored by semantic positioning on the X-axis, size of bullet points by significance ($\log_{10}$ of *P*-value). Due to space limitations, only the most significant terms are shown in **C**.

Enriched *cis*-regulatory elements in promoters of meiocyte up-regulated genes

Following up on transcriptional regulation, we extended our analysis to *cis*-regulatory elements/promoter motifs in genes up-regulated in *Arabidopsis* and maize meiocytes. Previously, we identified genes up-regulated in *Arabidopsis* meiocytes and confirmed the activity of their promoters in different stages of meiocytes with a Green Fluorescent Protein (GFP)-fusion approach (Li et al., 2012). Other studies also examined *cis*-regulatory elements, for example in sperm cells (the generative

Table 1
Transcription factors up-regulated in meiocytes in both *Arabidopsis* and maize

Arabidopsis gene ID	Description	Transcription factor family	Corresponding maize gene ID	Description	Transcription factor family
AT1G02030	C2H2-like zinc finger protein	C2H2			
AT1G06170	Transcription factor bHLH89	bHLH	AC233960.1_FG005	Helix-loop-helix DNA-binding	[a]
AT1G69180	Protein CRABS CLAW	C2C2-YABBY			
AT1G69490	NAC domain-containing protein 29	NAC			
AT2G16910	Transcription factor ABORTED MICROSPORES	bHLH	GRMZM2G139372	Helix-loop-helix DNA-binding	bHLH
AT2G31210	Transcription factor bHLH91	bHLH	AC233960.1_FG005	Helix-loop-helix DNA-binding	[a]
AT2G31220	Transcription factor bHLH10	bHLH	GRMZM2G021276		bHLH
AT2G36270	Protein abscisic acid-insensitive 5	bZIP	GRMZM2G168079	Basic-leucine zipper (bZIP) transcription factor	bZIP
AT2G42830	AGAMOUS-like MADS-box protein AGL5	MADS	GRMZM2G359952	Transcription factor, MADS-box	[a]
AT3G28470	myb proto-oncogene protein	MYB			
AT3G54340	Floral homeotic protein APETALA3	MADS	GRMZM2G139073	Transcription factor, MADS-box	MADS
AT4G18960	(AGAMOUS), MADS-box transcription factor	MADS	GRMZM2G010669	Transcription factor, MADS-box	[a]
AT5G06839	bZIP transcription factor-like protein	bZIP	GRMZM2G006578	Basic-leucine zipper (bZIP) transcription factor	bZIP
AT5G17800	myb domain protein 56	MYB			
AT5G19790	Ethylene-responsive transcription factor RAP2-11	AP2-EREBP	GRMZM2G384386	Pathogenesis-related transcriptional factor/ERF, DNA-binding	[a]
AT5G20240	Floral homeotic protein PISTILLATA	MADS	GRMZM2G110153	Transcription factor, MADS-box	MADS
AT5G60910	Agamous-like MADS-box protein AGL8	MADS	GRMZM2G147716	Transcription factor, MADS-box	MADS
			GRMZM2G139372	Helix-loop-helix DNA-binding	bHLH
			GRMZM2G384386	Pathogenesis-related transcriptional factor/ERF, DNA-binding	[a]
			GRMZM2G476357	Transcription factor TFIIB related	[a]

[a] No gene family annotated in the database.

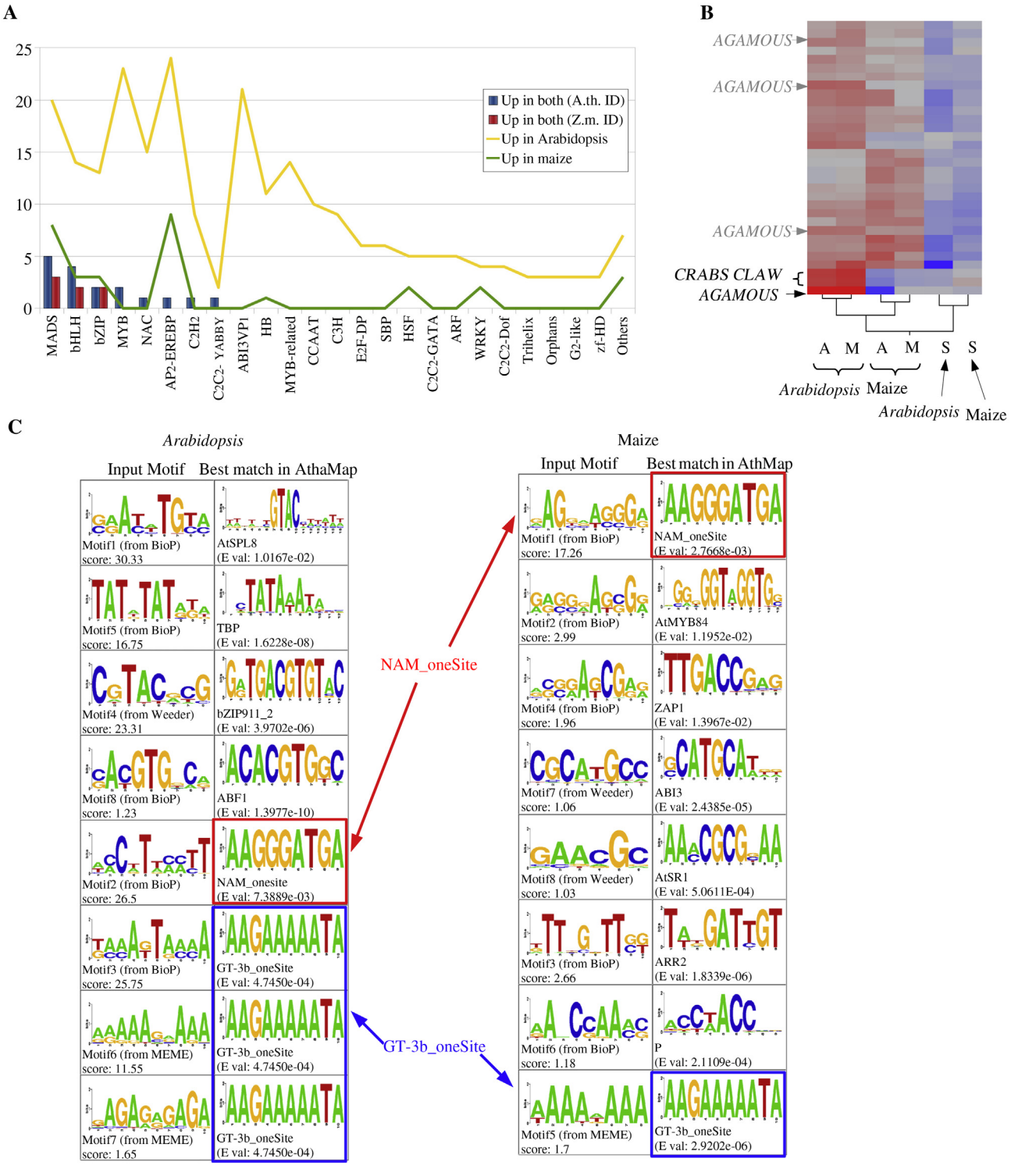

Fig. 4. Transcription factors and promoter motifs enriched in meiocytes.

A: Amount of annotated transcription factors in meiocyte-up-regulated genes. Bars show transcription factors with up-regulated orthologs in both organisms, while lines show transcription factors found significantly up-regulated in only *Arabidopsis* or maize. **B:** Expression heatmap of transcription factors. Some known *Arabidopsis* transcription factors have multiple maize orthologs, e.g., as pointed out CRABS CLAW. The annotated maize transcription factor related to AGAMOUS does not show similar expression (black), three other orthologs are candidates (grey). **C:** Promoter motifs found in genes co-up-regulated in *Arabidopsis* and maize. Sequence motifs enriched in promoter regions are shown in the left column of each panel, connected with the best known *cis*-regulatory element from the AthaMap database in the right columns. Arrows point to motifs found by analysis *via* both species.

cells of mature pollen) in both rice and *Arabidopsis* (Engel et al., 2005; Sharma et al., 2011). In this study, we used Promzea (http://promzea.org, Liseron-Monfils et al., 2013) to identify promoter DNA motifs associated with up-regulation in meiocytes in both *Arabidopsis* and maize. For most enriched *cis*-regulatory elements predicted in *Arabidopsis*, we observed higher Mean Normalized Conditional Probability (MNCP) scores than for those in maize (Fig. 4C), with everything >1 non-random, and the higher the more specific. The motifs for *Arabidopsis* also showed repeated occurrence, linking three detected adenine-enriched motifs with the known motif GT-3b_oneSite (Fig. 4C). The GT-3b_oneSite motif as well as the NAM_oneSite motif were detected in both *Arabidopsis* and maize (Fig. 4C). The identified motifs show both similarities and differences between *Arabidopsis* and maize, similar to the transcription factor analysis.

Mitochondrial genes are up-regulated in meiocytes

Our previous analyses of early meiocytes in maize and of various meiocyte stages in *Arabidopsis* pointed to high expression levels of transcripts derived from mitochondrial genes. In *Arabidopsis*, this was thought to be due to high expression of a part of the 620 kb large mitochondrial genome insertion (MGI) (Lin et al., 1999; Stupar et al., 2001) on chromosome 2 (Chen et al., 2010). In maize, MGIs/NUMTs (nuclear mitochondrial DNA sequences) are also frequent, but seem not to be contained in the reference genome (Lin et al., 1999; Clifton et al., 2004; Lough et al., 2008). Thus reads from mitochondrial transcripts only mapped to the gene on the mitochondrial genome itself, and we confirmed an up-regulation of genes whose products are needed for mitochondria function − including both nuclear-encoded and mitochondrial-encoded genes (Dukowic-Schulze et al., unpublished data). We now reassessed the origin of the transcripts reported for the chromosome 2 MGI with the data from early meiocytes in *Arabidopsis*.

Manually looking through the aligned reads (with Tablet Viewer), we found many single-nucleotide polymorphisms (SNPs) which at a closer look turned out to be C→U editing events (Table 2). This RNA editing is characteristic for RNA processing in mitochondria. Table 2 lists genes on the MGI in order, together with their mitochondrial counterparts, amount of edited events and reads in RPM. TEs within the MGI do not have any aligned reads, uncharacterized proteins have few, and the most reads are present for genes encoding components for

Table 2
MGI (mitochondrial genome insertion) genes on Chr. 2 in *Arabidopsis*

MGI gene ID	Mitochondrial gene ID	Editing event	Meiocyte RPM	Anther RPM	Seedling RPM	Description
AT2G07674	ATMG01010	0	11.95	2.36	0.25	Uncharacterized protein
AT2G07751	ATMG00990	11	27.92	8.66	0.50	NADH dehydrogenase I subunit 3
AT2G07675	ATMG00980	8	32.38	10.58	0.50	Ribosomal protein S12
AT2G07767		0	0	0	0	Transposable element gene
AT2G07676	ATMG00970	0	1.73	0.26	0.09	Uncharacterized protein
AT2G07768	ATMG00960	6	4.38	0.40	0.09	Cytochrome C assembly protein
AT2G07769		0	0	0	0	Transposable element gene
AT2G07669	[a]	0	1.18	0.06	0.02	Uncharacterized protein
AT2G07681	ATMG00900	22	23.30	4.23	0.57	Putative cytochrome c biosynthesis ccmC-like mitochondrial protein
AT2G07682		1	0	0	0	Transposable element gene
AT2G07772	ATMG00820	0	3.03	0.16	0.02	Uncharacterized protein
AT2G07683		0	0	0	0	Transposable element gene
AT2G07774	[a]	0	9.05	0.36	0.52	Uncharacterized protein
AT2G07685		0	0	0	0	Transposable element gene
AT2G07686		0	0	0	0	Transposable element gene
AT2G07687	ATMG00730	11	29.91	1.20	0.16	Cytochrome c oxidase subunit 3
AT2G07792	[a]	0	1.87	0.11	0.04	Pre-tRNA
AT2G07689	ATMG01320	11	14.10	0.49	0.25	NADH-Ubiquinone/plastoquinone (complex I) protein
AT2G07695	ATMG01280	3	11.60	0.71	0.32	Cytochrome c oxidase subunit II
AT2G07785	ATMG01275	4	9.31	0.48	0.21	NADH dehydrogenase I subunit 1
AT2G07698	ATMG01190	4	176.20	7.02	4.10	F-type H^+-transporting ATPase subunit alpha
AT2G07708	ATMG00500	4	39.86	2.51	0.65	Uncharacterized protein

[a] Not annotated, but sequence present.

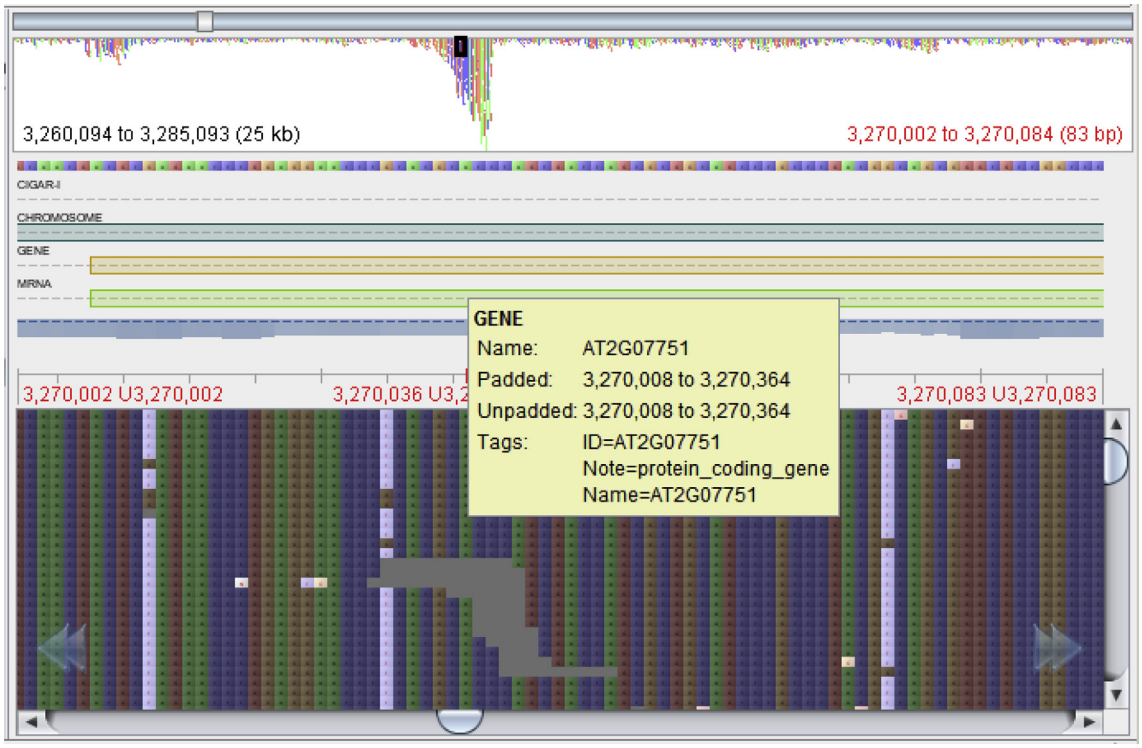

Fig. 5. Editing of mitochondrial genes.
The upper portion of this Tablet Viewer screenshot represents 25 kb of the mitochondrial DNA, framing a 1.1 kb area which is magnified below. The lower portion shows aligned reads to At2g07751, white squares represent C→U editing events.

the mitochondrial electron transport chain (NADH dehydrogenase subunits, Cytochrome C biosynthesis, ATPase). Example genes with reads and edited events are shown in Fig. 5. In addition, there are intergenic regions with many reads and editing events between the annotated genes on the MGI. To unequivocally distinguish between mitochondria genome and mitochondrial genome insertion in the nuclear genome, we followed a strategy used before by Adamo et al. (2008): at sites of known polymorphisms between *At2g07715* (nuclear) and *AtMg00560* (the mitochondrial *RPL2* gene), no reads were aligned for *At2g07715* due to the mismatch, but many aligned to the respective site in *AtMg00560* (results not shown). The editing site in this gene reported by Adamo et al. (2008) and Giége and Brennicke (1999) was not clearly detectable in our data and might be a site of rare or incomplete editing; two candidate sites in the vicinity had only 2 out of ~60, or 9 out of 38 nucleotides changed from C to U.

DISCUSSION

A previous study using isolated *Arabidopsis* meiocytes has analyzed the presence of orthologs of genes expressed in *Arabidopsis* meiocytes in different species, showing high occurrence of them in rice and poplar, and lower in mammals and yeast (Yang et al., 2011). They also found highly conserved single-copy genes, enriched for functions in housekeeping, DNA repair and replication (Yang et al., 2011). In this study, we evaluate the differences and similarities between meiocyte transcriptomes of *Arabidopsis* and maize, based on data from our lab, generated with the same experimental approach. The proportion of annotated genes decreases in both *Arabidopsis* and maize when going from seedlings to anthers to meiocytes. Unannotated features show an opposite pattern, and are the most frequent in meiocytes. This finding highlights that there is still a lot going on in meiosis which is unknown and has not yet been revealed by any studies done on the whole-plant level. Though the latest genome releases for maize contains 39,249 protein-coding genes, *vs*. 33,692 genes for *Arabidopsis*, the number of genes expressed above 10 RPM were very similar. However, when looking at genes differentially expressed, differences between *Arabidopsis* and maize became obvious, which also showed in our analysis *via* orthologous genes.

Differences and similarities between the *Arabidopsis* and maize meiocyte transcriptomes

The overall expression profile of putative orthologs showed high divergence between *Arabidopsis* and maize. However, similarities can also be found, especially regarding specific gene classes. Functional annotation analysis of genes up-regulated in meiocytes showed not only a high deviation in the number of significant processes (many more in *Arabidopsis* than in maize), but also in the kind of significant processes, even when using respective ortholog gene IDs. However, we caution about the interpretation of this matter since it might just be due to different quantity and quality of

annotations. But using both approaches − *via Arabidopsis* and maize gene IDs − might enhance the results obtained: On the one hand, analysis *via Arabidopsis* IDs confirmed that the meiocyte-up-regulated genes are mostly related to reproductive processes (flower development and meiosis), probably because so many genes involved in these processes are known, characterized and annotated in *Arabidopsis*. On the other hand, analysis *via* maize gene IDs resulted in far fewer and broader terms like metabolism or biosynthesis, indicating that maize gene annotations are not as extensive as in *Arabidopsis*, and also pointing to changes in the larger picture which may be masked in the *Arabidopsis* analysis by all the accumulating detail.

Thus, the differences detected in this direct one-to-one comparison between *Arabidopsis* and maize transcriptome data should not be considered as absolutely conclusive. Rather, concentrating on similarities found between the species will lead to more valuable and true biologically-relevant findings.

Meiotic genes

Of great interest to meiosis research is learning more about known meiotic genes and identifying new meiotic gene candidates for further studies. Two previous *Arabidopsis* studies of RNA-seq or microarray data from mixed meiocyte populations looked at common expression patterns of known meiotic genes. Chen et al. (2010) found that most known meiotic genes expressed at either more than 2-fold in meiocytes *vs*. anthers, or not more than 4-times higher in anthers than in meiocytes if expression in seedlings was less than half that of both meiocytes and anthers. Libeau et al. (2011) succeeded in clustering a few known meiotic genes together. A study in rice also showed that meiosis-specific pathways were enriched in PMCs (pollen mother cells = male meiocytes), but already so premeiotically, without much transcriptional change during meiosis (Tang et al., 2010). Identification of genes involved in sporo- and gametogenesis was undertaken in other large-scale studies and genes with expression in gametophytic cells were detected (Sundaresan et al., 1995). In the present study, we used orthologous gene pairs from *Arabidopsis* and maize for hierarchical clustering, and obtained a high enrichment for known meiotic genes in certain clusters. More than 80% of the known meiotic genes preferentially expressed in meiocytes could be found in one cluster, and even after applying more stringent clustering, still more than 40% of the genes clustered together, opening up a list of co-expressed genes as future meiotic gene candidates. While orthologous gene expression heatmaps did not show a lot of congruence between *Arabidopsis* and maize, a heatmap of only known meiotic genes demonstrated the tendency for co-up-regulation in meiocytes (and anthers) in *Arabidopsis* and maize. Prominent examples of this are the cyclin-encoding *SDS* (*SOLO DANCERS*) which is required for normal recombination and bivalent formation (Azumi et al., 2002; Wang et al., 2004), and *DMC1* (*DISRUPTION OF MEIOTIC CONTROL 1*) which is essential for meiotic recombination (Couteau et al., 1999; Kurzbauer et al., 2012).

Transcription factors and promoters in genes up-regulated in meiocytes

Known transcription factors involved specifically in meiosis are rare, the only meiotic transcription factor well-described in *Arabidopsis* is MMD1 (Male Meiocyte Death 1) (Yang et al., 2003). In our ortholog cluster data, MMD1 is connected to maize *GRMZM2G100629*, and pointed out as a meiotic gene in Fig. 2D. Interestingly, MMD1 was not detected in our analysis for transcription factors contained in the GO terms for transcription factor/regulator activity. To capture it, analysis would have had to be extended to more lenient GO terms like transcription and regulation of transcription. We found that well-known transcription factors involved in flower development (APETALA3, PISTILLATA, AGAMOUS) were up-regulated in meiocytes of both *Arabidopsis* and maize (Table S2). Taking a closer look at putative maize orthologs from *AtAGAMOUS* revealed four candidates; although three of them (*GRMZM2G359952*, *GRMZM2G052890*, and *GRMZM2G471089*) did not come up in our analysis for annotated transcription factors, they show far better meiocyte-up-regulated expression than the annotated candidate (*GRMZM2G010669*). This clearly shows that it is important to analyze data with different approaches and that computationally annotated orthologs have to be evaluated. Other known transcription factors present in the list of transcription factors up-regulated in meiocytes *vs*. seedlings are AMS (ABORTED MICROSPORES), involved in tapetal development and CRC (CRABS CLAW), involved in carpel development (Bowman and Smyth, 1999; Sorensen et al., 2003). These might not have direct roles for male meiosis, or their more severe mutant phenotypes regarding other reproductive parts may mask their importance and task in meiosis. Interestingly, transcription factors that seem to be up-regulated in meiocytes in only *Arabidopsis* or maize show a similar distribution of families: Beside MADS-box and bHLH transcription factors which are also high in the overlap data, AP2-EREBP transcription factors show a high peak.

When we analyzed the promoters of genes co-up-regulated in meiocytes of *Arabidopsis* and maize, clear similarities could be detected, but also differences showed up. The most significant motif found in only *Arabidopsis*, the AtSPL8-like motif, is the putative binding site of an SBP-box protein SQUAMOSA-PROMOTER-BINDING-PROTEIN-LIKE8 (AtSPL8), a regulator of sporogenic tissues (Unte et al., 2003; Xing et al., 2010). The most significant motif found in maize, the NAM_oneSite motif, was also identified in *Arabidopsis* with even three hits. The NAM_oneSite motif is the putative binding site of NAM, a transcription factor with a NAC domain. We also found NAC transcription factors in our transcription factor analysis in *Arabidopsis*, but not in maize, perhaps due to missing annotations. AtNAM is involved in the development, and mutants cannot develop shoot apical meristems, shoots and leaves (Duval et al., 2002). The other motif found in both species is the GT3-b_oneSite. GT elements are targeted by special trihelix DNA-binding factors, and GT3-a and GT3-b have been shown to be predominantly expressed

in floral buds and roots (Ayadi et al., 2004). Interestingly, motifs found in both species point to a link with other tissues that have high activity of cell division.

Connections to previous studies

The number of genes detected as expressed did not differ greatly from previous studies. Microarray analyses seem to detect fewer meiotic genes than expected, because of their bias towards sporophytic genes, *e.g.* on the Affymetrix ATH1 chip (Schmidt et al., 2012). In yeast, high-quality transcriptomic data showcased around 250 meiosis-specific genes (Mata et al., 2002). In *Arabidopsis* mixed meiocytes more than 800 (Yang et al., 2011) or more than 1000 (Chen et al., 2010) were detected as preferentially expressed in meiocytes. Since both *Arabidopsis* studies used the same plant material and collection method, the difference between the two studies reveals how much can depend on the experimental platform and data analysis.

Expression of transposable elements was detected in the two *Arabidopsis* studies. Yang et al. (2011) found around 5% TEs transcribed that were positively correlated with the transcription of neighboring genes, and Chen et al. (2010) detected ~1000 TEs up-regulated in meiocytes, located preferentially pericentromerically. However, we did not detect elevated TE activity in our new *Arabidopsis* dataset, and maize TEs were expressed but did not show a high up-regulation of specific TEs in meiocytes (Dukowic-Schulze et al., unpublished data).

Detecting mitochondrial transcripts in microarray or RNA-seq analyses where polyA selection was used is not often reported, due to discarding the mitochondrial transcripts as artifacts or not including the mitochondrion in the reference assembly. In the previous *Arabidopsis* meiocyte studies, one (Yang et al., 2011) did not explicitly mention any mitochondria genes up-regulated in meiocytes but listed the 18 most enriched PFAM families including Mito-Carr (Mitochondrial carrier), and TPR_1 and TPR_2 (Tetratricopeptide repeats) which can be found in the NADPH oxidase subunit and as receptor of mitochondrial import proteins. The other *Arabidopsis* study reported an increase of transcripts from mitochondria origins and attributed it to a mitochondrial genome insertion (MGI) because the reads mapped to there (Chen et al., 2010). We give the issue of mitochondrial importance for meiosis further thought in Dukowic-Schulze et al. (unpublished work, based on maize RNA-seq data). We want to point out that the detection of mitochondrial transcripts indeed originated in both species from mitochondria themselves, and that this should not be neglected.

There are also other transcriptome datasets of isolated meiocytes from different plant species available, such as rice (Suwabe et al., 2008; Tang et al., 2010; Kubo et al., 2013), or from different laboratories (Libeau et al., 2011; Yang et al., 2011; Schmidt et al., 2012). Because they are not based on the same stages, gender and platforms, we did not include them in our comparative analysis, though they can be a valuable source for future further examination of specific pathways and genes found in our present study.

Though the steps and processes of early meiosis in *Arabidopsis* and maize are quite similar, the overall expression profiles show broad differences. Similarities however can be found regarding the number of genes expressed, high transcript levels of mitochondrial genes and conserved transcriptional regulation. Most interesting and hopefully providing new candidates for further studies are the common clustering of meiotic genes as well as the transcription factors and *cis*-regulatory elements.

MATERIALS AND METHODS

Sample collection and processing

Meiocytes from both *Arabidopsis* and maize were collected with the CCM method (Chen and Retzel, 2013). Samples were processed for RNA extraction, library preparation, Illumina sequencing and read alignment as described previously (Chen et al., 2010) for *Arabidopsis* or slightly modified for maize.

Data analysis

Unique read counts were directly used for detection of differentially expressed genes with the DEseq package for *R* Statistical Analysis (Anders and Huber, 2012), and also transformed into RPM (reads per million mapped reads) for Venn diagrams. Genes overlapping between tissues were calculated with Excel, and verified using Venny (http://bioinfogp.cnb.csic.es/tools/venny/index.html). Expression heatmaps were generated with JMP Genomics, using the Ward method for hierarchical clustering of $\log_2$ transformed, normalized read count. For this and the DEseq analysis, data was trimmed to the upper 70% quantile of expressed genes.

For analysis of orthologous gene pairs, a homolog list was retrieved from the Biomart Tool on the Gramene webportal (http://gramene.org/). Statistics with DEseq were conducted on all gene pairs with read counts in both species (43,388 out of 48,256 orthologous gene pairs listed) trimmed to the upper 70% quantile (30,317 gene pairs). Many genes occurred at least as duplicates in the list, with all gene entries predicted as protein-coding.

Further analysis of genes up-regulated in meiocytes was based on a DEseq run with the ortholog data. For an adjusted *P*-value (Hochberg and Benjamini, 1990) of <0.01, 365 genes up-regulated in the simultaneous/ortholog analysis were found, *vs.* 4728 genes (*Arabidopsis*) or 2047 genes (maize) in separate analyses. Overlapping this data resulted in 227 genes up-regulated in meiocytes under all conditions. These were used for the examination of GO analysis with AgriGO (http://bioinfo.cau.edu.cn/agriGO/, Du et al., 2010) and Revigo (http://revigo.irb.hr/, Supek et al., 2011), and also for transcription factor and promoter analysis. Transcription factors defined by AgriGO were used further, and family annotations were taken from a Plant Transcription Factor Database (http://plntfdb.bioetanol.cnpem.br/, Riaño-Pachón et al., 2007). For promoter analyses, *cis*-regulatory motifs in the promoter regions for meiocyte-up-regulated *Arabidopsis* genes and maize

genes were predicted by Promzea (http://promzea.org, Liseron-Monfils et al., 2013). 500 bp long promoter regions were analyzed and predicted motifs were compared to known promoter motifs in the AthaMap database (Steffens et al., 2004; Galuschka et al., 2007) using STAMP (Mahony and Benos, 2007).

Manual read alignment examination for mitochondria-related transcripts was conducted with Tablet Viewer (Milne et al., 2012).

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SUPPLEMENTARY DATA

Table S1. Number of unique counts aligned for all samples.

Table S2. Transcription factors up-regulated in meiocytes of *Arabidopsis* and maize.

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jgg.2013.11.007.

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